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Environmental Investigations of *Vibrio parahaemolyticus* in Oysters after Outbreaks in Washington, Texas, and New York (1997 and 1998)

ANGELO DEPAOLA,[1]* CHARLES A. KAYSNER,[2] JOHN BOWERS,[3] AND DAVID W. COOK[1]

Gulf Coast Seafood Laboratory, Food and Drug Administration, Dauphin Island, Alabama 36528[1]; Seafood Products Research Center, Food and Drug Administration, Bothell, Washington 98021[2]; and Division of Mathematics and Statistics, Food and Drug Administration, Washington, D.C. 20204[3]

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Total *Vibrio parahaemolyticus* densities and the occurrence of pathogenic strains in shellfish were determined following outbreaks in Washington, Texas, and New York. Recently developed nonradioactive DNA probes were utilized for the first time for direct enumeration of *V. parahaemolyticus* in environmental shellfish samples. *V. parahaemolyticus* was prevalent in oysters from Puget Sound, Wash.; Galveston Bay, Tex.; and Long Island Sound, N.Y., in the weeks following shellfish-associated outbreaks linked to these areas. However, only two samples (one each from Washington and Texas) were found to harbor total *V. parahaemolyticus* densities exceeding the level of concern of 10,000 g^{-1}. Pathogenic strains, defined as those hybridizing with *tdh* and/or *trh* probes, were detected in a few samples, mostly Puget Sound oysters, and at low densities (usually <10 g^{-1}). Intensive sampling in Galveston Bay demonstrated relatively constant water temperature (27.8 to 31.7°C) and *V. parahaemolyticus* levels (100 to 1,000 g^{-1}) during the summer. Salinity varied from 14.9 to 29.3 ppt. A slight but significant ($P < 0.05$) negative correlation (−0.25) was observed between *V. parahaemolyticus* density and salinity. Based on our data, findings of more than 10,000 g^{-1} total *V. parahaemolyticus* or >10 g^{-1} *tdh*- and/or *trh*-positive *V. parahaemolyticus* in environmental oysters should be considered extraordinary.

Vibrio parahaemolyticus is a gram-negative halophilic bacterium distributed in temperate and tropical coastal waters throughout the world and is a leading cause of foodborne gastroenteritis (15). Until recently, U.S. illnesses were limited to sporadic cases associated with consumption of raw shellfish (12, 13) or with small outbreaks due to recontamination of cooked or processed seafood (3).

During the summer of 1997, the first confirmed oyster-associated outbreak caused by *V. parahaemolyticus* in the United States, as defined by the National Shellfish Sanitation Program, occurred in the Pacific Northwest (5). During this outbreak, 209 culture-confirmed cases were reported in North America, and nearly all were associated with shellfish from Washington and British Columbia. Multiple serotypes of *V. parahaemolyticus* were isolated from the stools of ill persons. A smaller oyster-associated outbreak (43 culture-confirmed cases) occurred in Washington in 1998 (Ned Therien, Washington State Department of Health, personal communication, February 1999 and February 2000).

The largest *V. parahaemolyticus* outbreak reported in the United States (416 cases, 98 culture confirmed) was linked to consumption of raw oysters from Galveston Bay, Tex. (7; S. S. Barth, L. S. Del Rosario, T. Baldwin, M. Kingsley, V. Headley, B. Ray, K. Wiles, A. DePaola, D. Cook, C. Kaysner, N. Puhr, N. Daniels, L. Kornstein, and M. Nishibuchi, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., abstr. C-57, 1999). This outbreak lasted from May to July 1998 and was distinguished by an extremely high attack rate and by the fact that all clinical isolates belonged to a single clone of the O3:K6 serotype. This clone apparently emerged in India around 1995, becoming endemic in much of Asia; it is the most prevalent strain associated with *V. parahaemolyticus* illness in Asia (2, 22). It appears that this O3:K6 clone has become pandemic, and there is concern that this may increase the risk of *V. parahaemolyticus* infections from consumption of U.S. shellfish.

V. parahaemolyticus O3:K6 was subsequently linked to a small outbreak of eight *V. parahaemolyticus* cases associated with shellfish harvested from Oyster Bay off New York's Long Island Sound from July to September 1998 (6). In each outbreak, state health officials closed affected areas to shellfish harvesting and requested the assistance of the U.S. Food and Drug Administration (FDA) with monitoring of shellfish for abundance of *V. parahaemolyticus*. Areas in Galveston Bay remained closed until November based on historical seasonal epidemiological data.

The distribution of *V. parahaemolyticus* in the environment and foods has been studied extensively in Japan (19, 27) and to a lesser degree in the United States (9, 16, 17, 18, 25). It is found in Pacific, Gulf, and Atlantic coastal waters and fauna, but there are few quantitative data on seasonal or geographical distribution. A nationwide survey of shellfish and overlying waters was conducted in 1983, but sampling frequency was only once per season for 1 year (9). Levels in shellfish were found to be 200-fold higher than in overlying waters; the highest densities were observed in the late spring and early summer. Water temperature was positively correlated with *V. parahaemolyticus* abundance, but no clear relation was observed with salinity or fecal coliform levels. The amount of sampling was limited by the available methodology. The most-probable-number (MPN) procedure, which relies on biochemical identification of suspect isolates (11), is laborious and expensive.

Pathogenic strains of *V. parahaemolyticus* generally produce a thermostable direct hemolysin (TDH) that is associated with

* Corresponding author. Mailing address: Gulf Coast Seafood Laboratory, U.S. Food and Drug Administration, Dauphin Island, AL 36528-0158. Phone: (334) 694-4480 (ext. 230). Fax: (334) 694-4477. E-mail: adepaola@cfsan.fda.gov.

the Kanagawa phenomenon (K^+) and/or a TDH-related hemolysin (TRH) (14). The genes *tdh* and *trh* code for TDH and TRH, respectively; the *tdh* gene has been used as the target of DNA probes (9). One or both of these genes are detected in most clinical strains of *V. parahaemolyticus* but are uncommon in environmental and food isolates (9, 17, 27). All strains of *V. parahaemolyticus* produce a thermolabile direct hemolysin, which reportedly is species specific (24). Recently, alkaline phosphatase- and digoxigenin-labeled oligonucleotide probes for detection of *tlh* were evaluated, and their results were shown to be in agreement with standard biochemical identification assays (20). Replacement of biochemical tests for bacterial identification with DNA probe hybridization substantially reduces the time and labor required for sample analysis. *Vibrio vulnificus* enumeration by DNA probe identification of colony lifts from direct plating of oyster homogenates was equivalent to MPN analysis and more rapid and precise (10), but this approach has not been reported for *V. parahaemolyticus*.

This paper reports total *V. parahaemolyticus* densities and the occurrence of pathogenic strains in shellfish following outbreaks in Washington, Texas, and New York. Recently developed nonradioactive DNA probes were utilized for the first time for direct enumeration of *V. parahaemolyticus* in environmental shellfish samples.

MATERIALS AND METHODS

Sample collection. In Washington State, samples were obtained from commercial growers that harvested shellfish from 20 August to 3 September 1997. Samples were collected at wholesale (23 samples consisting of 12 shellstock oysters each) and retail (two samples of shellstock oysters and five of shucked oyster meats) markets by the Washington State Department of Health. Four samples of wholesale market shellstock oysters were collected from oysters harvested in Oregon waters on 26 August 1997. Ten wholesale market samples of shellstock oysters were collected on the date of harvest in Washington from 10 to 17 August 1998. Data on water temperature and salinity at the harvest sites were not available. Samples were cooled with ice bricks during transport and storage and analyzed within 24 h of collection at the FDA district laboratory in Bothell, Wash.

Three to five Galveston Bay sites (private oyster leases) were sampled by the Texas Department of Health generally at weekly intervals from 29 June to 21 September 1998. From 17 August to 8 September 1998, samples were collected from all 30 leases that were active during the outbreak period on the weeks of 17 and 24 August. Twenty of the leases were sampled on the week of 31 August, and 10 were sampled on 8 September. Bottom-water temperature and salinity were determined at each sample site by using a YSI model 30 salinity meter (YSI, Yellow Springs, Ohio). Samples consisting of 12 shellstock oysters were collected and immediately cooled by placing bagged ice on top of the oysters; bubble wrap was placed between the ice and the oysters to insulate the oysters. The chilled oysters were placed in insulated containers with ice bricks and shipped to the FDA laboratories in Dauphin Island, Ala.; Atlanta, Ga.; or Denver, Colo., for bacterial analysis. Samples were analyzed within 24 h of collection. Samples warmer than 13°C were excluded from the data analysis.

In New York, duplicate or triplicate samples (12 shellstock oysters each) were collected from each of three sites in Oyster Bay on 12 and 14 October 1998 by the New York State Department of Environmental Conservation. Temperature and salinity were determined using a YSI model 30 salinity meter. Sample handling and shipment were as described for the Texas samples except that all were analyzed at the FDA laboratory in Dauphin Island, Ala.

Bacterial analysis. The MPN procedure described in the FDA's Bacteriological Analytical Manual (BAM) (11) was used to determine total *V. parahaemolyticus* density in the 1997 Washington and Oregon samples and in a small portion (16 of 106 samples) of the Texas samples. Suspect isolates were identified by the API 20E system (bioMerieux Vitek, Inc., Hazelwood, Mo.) and/or by hybridization with alkaline phosphatase- and digoxigenin-labeled *tlh* oligonucleotide probes (20). Production of urease was determined as described in the BAM (11). Suspect colonies were also screened for hybridization with a digoxigenin-labeled *tdh* probe, and Washington isolates were tested with a digoxigenin-labeled *trh* probe. Isolates hybridizing with either probe were tested for TDH production by the Kanagawa assay (11). Digoxigenin-labeled probe, filter preparation, hybridization, and chromogenic detection were done as described by the manufacturer (Genius System user's guide for filter hybridization, version 2.2-92; Boehringer Mannheim Corp., Indianapolis, Ind.) and Weagant et al. (28). The *tdh* and *trh* probes were synthesized using primers and PCR according to Nishibuchi et al. (21) and Bej et al. (4).

Total and pathogenic *V. parahaemolyticus* densities in 1998 Washington and New York samples and most (103 of 106) of the Texas samples were determined by spread plating and hybridization procedures using the *tlh* and *tdh* DNA probes described above. Oysters were scrubbed, shucked, and homogenized 1:1 in phosphate-buffered saline (PBS), and serial 10-fold dilutions were prepared in PBS using the recommended procedures of the American Public Health Association (1). With the Texas samples, 0.1, 0.01, and 0.001 g of oyster homogenates were spread plated without replication onto T_1N_3 agar (10.0 g of tryptone [Difco Laboratories, Detroit, Mich.], 30.0 g of NaCl, 20.0 g of Bacto agar [Difco], and 1.0 liter of deionized water) with incubation overnight at 35°C. With New York samples, two replicate 0.1-g portions were plated onto T_1N_3 agar for enumeration of total *V. parahaemolyticus*, and 10 replicate 0.1-g portions were plated onto T_1N_3 agar for identification of *tdh*-positive *V. parahaemolyticus*. Plates were incubated overnight at 35°C. For the Texas and New York samples, colony lifts were prepared on Whatman 541 filters, and hybridizations were performed as described for *V. vulnificus* (10) except that the *V. parahaemolyticus* alkaline phosphatase-labeled *tlh* probe was used and hybridization conditions were modified as recommended by McCarthy et al. (20). Total *V. parahaemolyticus* densities in the 1998 Washington samples were determined by plating onto nylon transfer membranes (MagnaGraph, 82 mm; Osmonics Inc., Westboro, Mass.) previously placed on T_1N_3 agar plates. After incubation at 35°C for 3 h, the filters were transferred to TCBS agar (Difco) plates and incubated overnight at 35°C. Colony lifts and hybridization with digoxigenin-labeled *tlh* probe were done as described above.

Direct plating for pathogenic *V. parahaemolyticus* in Texas and New York samples was also done on nylon transfer membranes, but the initial plating medium was tryptic soy agar (Difco) supplemented with 25.0 g of NaCl per liter and 1.5 g of $MgSO_4$ per liter to allow cell repair (8). After incubation at 35°C for 3 h, filters were transferred to thiosulfate-citrate-bile salts-sucrose (TCBS) agar and treated as described above except that hybridization was done with a digoxigenin-labeled *tdh* probe.

V. parahaemolyticus isolates from the West Coast that hybridized with either the *tdh* or *trh* probe were tested for somatic (O) serotype as described in the BAM (11). Clinical isolates from Texas were serotyped for somatic and capsular antigens by the Centers for Disease Control and Prevention.

Statistical methods. Sample and method error variation of total *V. parahaemolyticus* counts in New York samples were estimated by an analysis of variance. Plates which were indeterminate (nondetect) were assigned one half the limit of detection (5 CFU/g). Association between *V. parahaemolyticus* densities and salinity for the Texas samples was determined by Pearson correlation.

RESULTS

V. parahaemolyticus was recovered from most (77%) of the Pacific Northwest samples during the late summers of 1997 and 1998 (Table 1). Densities varied considerably (<3 to 46,000 g^{-1}) in Washington oysters over a short period from 20 August to 3 September 1997. Densities ranged from 29 to 2,300 g^{-1} on 26 August 1997 in different areas of Quilcene Bay. Similar variation was seen at the Twanoh State Park sampling site in Hood Canal from one week to the next. Strains with *trh* and/or *tdh* were detected at densities of <10 g^{-1} in 15% of the 1997 samples but were not detected in 1998 samples. All strains that were *tdh* positive were also K^+, *trh* positive, and urease positive; one strain was *trh* positive and *tdh* negative. Several O serotypes (serotypes 1, 4, and 5) were found among these potentially pathogenic strains.

V. parahaemolyticus was detected in all 106 Texas oyster samples, and counts ranged from 40 to 23,000 g^{-1}. *V. parahaemolyticus* densities in most samples ranged between 100 and 1,000 g^{-1} (15.1% were >1,000 g^{-1} and 9.5% were <100 g^{-1}). Figure 1 indicates little weekly variation in *V. parahaemolyticus* levels in Texas oysters except for 29 June 1998, when a density of 23,000 g^{-1} was found with one sample; this was the only sample from Texas that exceeded 10,000 g^{-1}. Mean *V. parahaemolyticus* densities at the three to five sites sampled throughout the study were similar to those observed with the expanded sampling set (20 to 30 sites) in August 1998. Greater variation was observed with the MPN analysis than with the direct plating procedure.

Table 2 shows *V. parahaemolyticus* levels in oysters and salinity at 30 Galveston Bay leases from 17 August to 8 September 1999. Approximately a one-log range (2.07 to 3.16) in mean ($\log_{10}$) *V. parahaemolyticus* densities was observed among the sites. Salinity ranged from 14.9 to 29.3 ppt. Water temperature

TABLE 1. U.S. West Coast oyster samples analyzed in 1997 and 1998 for *V. parahaemolyticus* levels

Harvest location[a]	Sample dates (mo/day/yr)			Sample type[b]	*V. parahaemolyticus*[c] (no. g^{-1})	
	Date harvested	Date collected	Date analyzed		Total	tdh and trh positive
Washington						
Elk R. Grays Harbor	8/25/97	8/25/97	8/26/97	W	120	<3
Elk R. Grays Harbor	9/03/97	9/03/97	9/04/97	W	430	<3[d]
Elk R. Grays Harbor		9/02/97	9/04/97	SR	93	<3
Wescott Bay, SJI	8/25/97	8/25/97	8/26/97	W	<3	<3
Ship Bay, Eastsound, SJI	8/26/97	8/26/97	8/27/97	W	<3	<3
Samish Bay, N. Puget Snd.	9/02/97	9/02/97	9/03/97	W	9.1	<3
Edison Slough, Samish Bay	9/02/97	9/02/97	9/03/97	W	<3	<3
Sequim Bay, N. Puget Snd.	8/26/97	8/26/97	8/27/97	W	<3	<3
Possession Snd, Tulalip, N. PS	8/26/97	8/26/97	8/27/97	W	230	<3
Pt. Julia, N. Puget Snd.	8/26/97	8/26/97	8/26/97	W	<3	<3
Dabob Bay, Hood Canal	8/20/97	8/25/97	8/27/97	R	150	<3
Dabob Bay, Hood Canal	8/20/97	8/26/97	8/27/97	R	<3	<3
Eagle Creek, Hood Canal	9/02/97	9/02/97	9/03/97	W	4,300	<3
Quilcene sec. A, Hood Canal	8/26/97	8/26/97	8/26/97	W	2,300	<3
Quilcene sec. A, Hood Canal	9/02/97	9/02/97	9/03/97	W	230	<3
Quilcene sec. J, Hood Canal	8/26/97	8/26/97	8/27/97	W	29	<3
Quilcene sec. J, Hood Canal	9/02/97	9/02/97	9/03/97	W	230	<3
Twanoh St. Park, Hood Canal	8/26/97	8/26/97	8/26/97	W	42	<3
Twanoh St. Park, Hood Canal	9/02/97	9/02/97	9/03/97	W	2,300	<3
Rocky Bay, S. Puget Snd.	8/25/97	8/25/97	8/26/97	W	46,000	<3
Rocky Bay, S. Puget Snd.		9/03/97	9/03/97	SR	<3	<3
Case Inlet, S. Puget Snd.	8/25/97	8/25/97	8/26/97	W	21	3.6
Eld Inlet, S. Puget Snd.		9/03/97	9/04/97	SR	43	<3
Eld Inlet, S. Puget Snd.		9/03/97	9/04/97	SR	93	7.3
Nisqually, S. Puget Snd.	8/23/97	8/25/97	8/26/97	W	39	<3
Totten Inlet, S. Puget Snd.	9/02/97	9/02/97	9/03/97	W	4,300	<3
Bay City, Willapa Bay		9/03/97	9/04/97	W	<3	<3
Stackpole, Willapa Bay		9/03/97	9/04/97	W	150	<3
Stackpole, Willapa Bay	9/01/97	9/03/97	9/04/97	SR	21	<3
Oysterville, Willapa Bay	8/25/97	8/25/97	8/26/97	W	<3	<3
Westcott Bay, SJI	8/10/98	8/10/98	8/11/98	W	300	<10
Dosewallips, Hood Canal	8/17/98	8/17/98	8/18/98	W	400	<10
Penn Cove, N. Puget Snd.	8/17/98	8/17/98	8/18/98	W	<100	<10
North Bay, Case Inlet	8/10/98	8/10/98	8/11/98	W	1,600	<10
Case Inlet, S. Puget Snd.	8/17/98	8/17/98	8/18/98	W	100	<10
Rocky Bay, S. Puget Snd.	8/17/98	8/18/98	8/18/98	W	700	<10
Hammersley Inlet, S. Puget Snd.	8/10/98	8/10/98	8/11/98	W	800	<10
N. Totten Inlet, S. Puget Snd.	8/17/98	8/17/98	8/18/98	W	500	<10
S. Totten Inlet, S. Puget Snd.	8/10/98	8/10/98	8/11/98	W	11,000	<10
Oregon						
Netarts Bay		8/26/97	8/27/97	W	7.3	<3
S. Slough, Coos Bay	8/22/97	8/26/97	8/27/97	W	230	3.6
Tillamook Bay	8/26/97	8/26/97	8/27/97	W	11	<3
Yaquina Bay	8/26/97	8/26/97	8/27/97	W	23	3.6

[a] Abbreviations: R., river; SJI, San Juan Island; Snd., Sound; PS, Puget Sound; Sec., Section; St., State; Pt., port.
[b] W, shellstock from wholesale market; R, shellstock from retail market; SR, shucked meats from retail market.
[c] See Materials and Methods. The 1997 counts were determined by the MPN procedure, and the 1998 counts were determined by the direct plating procedure.
[d] *trh* = 3.6.

varied little (27.8 to 31.7°C) and is not shown. Higher *V. parahaemolyticus* densities and lower salinities were found in the Smith Point and East Bay areas than in the Ship Channel and Hanna Reef areas. A slight but significant ($P < 0.05$) negative correlation (−0.25) was observed between salinity and $\log_{10}$ *V. parahaemolyticus* density. We recovered one *tdh*-positive isolate, serotype O4:K-untypeable. Two of the three samples collected on 21 September 1998 yielded colonies hybridizing with the *tdh* DNA probe, but no isolates were cultured for subtyping.

Table 3 lists densities of *V. parahaemolyticus* in Oyster Bay, N.Y., oysters and method variability. Densities ranged from <10 to 120 g^{-1} in oysters from various sites in Oyster Bay. There was no apparent trend in *V. parahaemolyticus* densities between sites or collection dates and little variation in temperature (17.4 to 17.8°C) or salinity (25.5 to 26.2 ppt) of the harvest waters. Strains hybridizing with the *tdh* probe were not detected. Total variance (total *V. parahaemolyticus* density determined by direct plating and identification by the alkaline phosphatase-labeled *tlh* probe) from sample to sample was 0.13 ($\log_{10}$), while method error was minimal (0.06).

DISCUSSION

This paper reports *V. parahaemolyticus* levels in oysters following four shellfish outbreaks and provides the most extensive

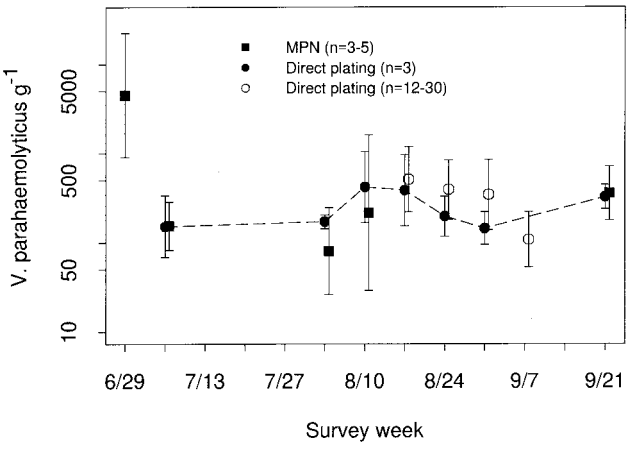

FIG. 1. Mean *V. parahaemolyticus* densities in Galveston Bay oysters obtained from 29 June through 21 September 1998. Symbols: ●, direct plating of three lease sites sampled throughout the study; ○, direct plating during August for the expanded sampling set of 30 lease sites; ■, MPN of three to five lease sites sampled throughout the study. Values are mean CFU per gram ± standard deviation. Dates are month/day.

quantitative data for *V. parahaemolyticus* in U.S. shellfish to date. *V. parahaemolyticus* was prevalent in oysters from Puget Sound, Wash.; Galveston Bay, Tex.; and Long Island Sound, N.Y., in the weeks following shellfish-associated outbreaks linked to these areas. *V. parahaemolyticus* densities exceeded the FDA level of concern of 10,000 g^{-1} (26) in two samples, one each from Washington and Texas. Pathogenic strains (those hybridizing with the *tdh* and/or *trh* probe) were detected in a few samples, mostly Puget Sound oysters, and at low densities (usually <10 g^{-1}). The isolation of K^+ strains from incriminated food or the environment associated with *V. parahaemolyticus* outbreaks has not been reported previously in the United States (3).

While much of the variation in *V. parahaemolyticus* levels in the present study may be attributed to seasonal and regional differences, the findings may also have been influenced by temporal or spatial proximity of samples to incriminated shellfish, sampling protocols, and bacteriological procedures for each outbreak. With the exception of Washington State, *V. parahaemolyticus* surveillance was not initiated until shellfish harvesting areas were closed. Washington State shellfish were linked to 68 illnesses from May to September 1997, with the peak period for onset of cases from 10 to 23 August (35 reported cases). Environmental monitoring began on 20 August 1997, prior to closure of oyster harvesting areas on 28 to 29 August 1997 by the Washington Department of Health; monitoring extended through 7 September 1997 (T. Sample and C. Swanson, *Vibrio parahaemolyticus* Workshop, U.S. Food and Drug Administration, 1997). While many of the samples were collected after closure of the harvest areas, the *V. parahaemolyticus* levels in these samples were similar to those in samples collected prior to closure near the peak of the outbreak. *V. parahaemolyticus* densities also varied more in Puget Sound shellfish than in those from Galveston Bay and Long Island Sound. Some shellfish-growing areas in the Puget Sound are exposed during low tide, which may elevate the temperature of emerged oysters, because air temperatures are typically much warmer than water temperatures in the late summer. Since *V. parahaemolyticus* growth is favored by warmer temperatures, it would probably multiply more rapidly in emerged oysters than in those submerged. In many instances, Washington and Oregon oyster samples were collected from harvesters at the dock or from retail markets; water temperature, salinity, water depth, precise harvest time, and postharvest handling data were not available, but these factors may influence *V. parahaemolyticus* levels and variability. Results from our laboratory indicate that *V. parahaemolyticus* densities can increase 100-fold in live oysters within 10 h of harvest at 26°C (J. A. Gooch, A. DePaola, C. A. Kaysner, and D. L. Marshall, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., abstr. P-52, 1999). MPN analysis was used for enumeration of *V. parahaemolyticus* in 1997 Washington samples and has been shown to be much less precise than direct plating for enumeration of *V. vulnificus* from oysters (10).

Levels of K^+ *V. parahaemolyticus* in this study were well below the infectious dose (10^5) observed in feeding experiments with human volunteers (23). The *V. parahaemolyticus* clinical strains from the Pacific Northwest are nearly always urease positive and usually have both the *tdh* and *trh* virulence genes. These strains may be more virulent than those used in early feeding experiments with human volunteers (23), described as K^+ without information on urease and *trh*. The low densities (<10 g^{-1}) in environmental and market oysters suggest that illness occurs in some individuals at doses well below the 10^5 to 10^7 range observed in feeding trials (23). The con-

TABLE 2. *V. parahaemolyticus* counts in oysters from Galveston Bay, Tex.[a]

Area	Lease code	Log (*V. parahaemolyticus*/g)	Salinity (ppt)
Ship Channel	4	2.38 ± 0.31	26.5 ± 2.46
	6	2.27 ± 0.08	26.9 ± 2.47
	7	2.80 ± 0.38	26.5 ± 2.25
	8	2.18 ± 0.30	26.2 ± 2.32
	9	2.09 ± 0.22	25.3 ± 1.74
	10	2.36 ± 0.47	22.2 ± 6.60
	11	2.07 ± 0.42	25.0 ± 1.65
	12	2.25 ± 0.39	23.3 ± 1.06
	13	2.63 ± 0.71	26.1 ± 1.91
	14	2.59 ± 0.65	26.0 ± 1.99
	15	2.19 ± 0.14	24.2 ± 1.65
	16	2.32 ± 0.18	26.1 ± 1.29
	27	2.50 ± 0.43	24.1 ± 0.61
	Total	2.36 ± 0.39	25.3 ± 2.52
Smith Point	5	2.87 ± 0.12	19.9 ± 0.35
	17	2.76 ± 0.11	19.1 ± 0.89
	18	2.56 ± 0.20	18.8 ± 0.76
	28	2.52 ± 0.48	19.4 ± 1.56
	29	2.59 ± 0.36	19.5 ± 1.56
	Total	2.67 ± 0.26	19.3 ± 0.90
East Bay	1	2.84 ± 0.20	21.0 ± 1.27
	2	3.16 ± 0.17	19.4 ± 1.33
	3	2.87 ± 0.18	22.5 ± 3.03
	21	2.98 ± 0.38	19.5 ± 1.70
	22	2.66 ± 0.44	19.9 ± 1.48
	23	2.47 ± 0.27	18.0 ± 4.10
	24	2.68 ± 0.86	21.7 ± 0.35
	25	2.88 ± 0.31	19.9 ± 1.57
	26	2.18 ± 0.33	21.1 ± 0.99
	30	2.60 ± 0.02	19.6 ± 0.29
	Total	2.73 ± 0.42	20.4 ± 1.93
Hanna Reef	19	2.53 ± 0.43	24.4 ± 1.53
	20	2.25 ± 0.19	22.6 ± 2.05
	Total	2.39 ± 0.33	23.5 ± 1.90

[a] *V. parahaemolyticus* and salinity values are means ± standard deviation of three samples collected from 17 August to 8 September 1998.

TABLE 3. *V. parahaemolyticus* counts in oysters from Oyster Bay Harbor, N.Y.

Sample no.-site	Date harvested (mo/day/yr)	No. of *tlh*-positive colonies/0.1 g		Total *V. parahaemolyticus*[a] (CFU g^{-1})
		Replicate A	Replicate B	
1-A	10/12/98	2	3	25
2-A	10/12/98	2	2	20
3-A	10/12/98	1	5	30
4-B	10/12/98	3	3	30
5-B	10/12/98	0	0	<10
6-B	10/12/98	2	2	20
7-C	10/12/98	1	2	15
8-C	10/12/98	9	15	120
9-A	10/14/98	5	4	45
10-A	10/14/98	3	5	40
11-A	10/14/98	2	0	10
12-B	10/14/98	3	3	30
13-B	10/14/98	3	2	25
14-C	10/14/98	0	3	15
15-C	10/14/98	1	1	10

[a] Mean total *V. parahaemolyticus* density per gram derived from replicate A and B samples.

tribution of postharvest temperature abuse in this outbreak was not determined and requires further study.

V. parahaemolyticus levels in Galveston Bay oysters were higher and less variable than those in Washington State oysters; the higher counts were probably due to warmer Gulf waters. The lower variability was attributed to nearly constant water temperature during the study and to careful sample handling procedures from harvest to analysis, as these samples were collected in the field and cooled immediately rather than collected from the market as in Washington. Unpublished results from our laboratory indicate that the cooling and shipping protocol used with the Texas and New York samples does not affect *V. parahaemolyticus* numbers in oysters from harvest to analysis within 24 h. Texas samples were analyzed by direct plating methods instead of MPN, further reducing variability. The only count that exceeded 10,000 g^{-1} was for a sample collected on 29 June 1998; relatively high densities from this harvest date were observed in two other samples (930 and 4,300 g^{-1}). The higher counts in these samples could reflect their closer temporal proximity to the time of incriminated-oyster harvest. Only three samples yielded colonies that hybridized with the *tdh* probe. Isolates were not cultured from two of the samples. A single isolate from the third sample was not the O3:K6 serotype associated with clinical samples. Harvest for raw consumption resumed in November 1998; no new cases were reported.

Oysters and clams from the Long Island Sound were incriminated in 16 *V. parahaemolyticus* cases from 21 July to 27 September 1998; O3:K6 was the predominant serotype (6). On 10 September 1998, the New York State Department of Environmental Conservation closed Oyster Bay to harvesting of shellfish. Samples from New York were collected on 12 October (8 samples) and 14 October (7 samples) 1998, more than 1 month after closure. The low *V. parahaemolyticus* densities (generally <100 g^{-1}) in these samples may reflect the cooling water temperatures (17°C). To increase sensitivity to 1 g^{-1}, 10 plates were spread with replicate 0.1-g portions of oyster homogenate from each sample. While no O3:K6 strains were detected, the low method variance with the New York samples did demonstrate the precision of the direct plating procedure.

This study demonstrates the abundance of *V. parahaemolyticus* in U.S. oysters following outbreaks on the Pacific, Gulf, and Atlantic coasts. Based on our data, findings of more than 10,000 total *V. parahaemolyticus* or >10 *tdh*- and/or *trh*-positive *V. parahaemolyticus* per g in environmental oysters should be considered extraordinary. Monitoring total and pathogenic *V. parahaemolyticus* levels during warmer periods of the year could provide valuable information on conditions leading to an outbreak and may be useful in forecasting outbreaks or developing reliable criteria for reopening shellfish beds after an outbreak. Analysis of incriminated market shellfish would help resolve questions regarding the possibility of increased strain virulence and the importance for risk assessment of postharvest multiplication.

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